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Production of Elastase, Exotoxin A, and Alkaline Protease in Sputa during Pulmonary Exacerbation of Cystic Fibrosis in Patients Chronically Infected by *Pseudomonas aeruginosa*

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Secretion of *Pseudomonas aeruginosa* elastase, exotoxin A, and alkaline protease in sputum during bronchopulmonary exacerbations was examined in 18 cystic fibrosis patients chronically infected with this microorganism. The patients were studied during one or several exacerbation periods necessitating hospitalizations of 12 to 20 days. In all cases, *P. aeruginosa* was present in bronchial secretions at admission and was not eradicated after treatment. The *P. aeruginosa* density decreased significantly after antibiotic therapy but remained greater than 10^6 CFU/g of sputum in most cases. Significant amounts of *P. aeruginosa* exoproteins were measured in total homogenized bronchial secretions by immunoenzymatic assays. The detection of higher levels of exoproteins at admission, the significant decrease after treament, and the absence of exoproteins during intercrisis phases constituted arguments for a renewal of virulence of *P. aeruginosa* during exacerbations. Nevertheless, the concomitant changes in bacteria load and the triggering of the inflammatory process and immune complex formation could also contribute to pulmonary exacerbations.

Pseudomonas aeruginosa is the bacterium most frequently associated with pulmonary infection in cystic fibrosis (CF) patients (15). It persists in the airways of CF patients despite intensive antibiotic therapy and contributes to pulmonary failure, which is the major cause of morbidity and mortality in CF patients. The infection is generally chronic, with persistence of the pathogen at the mucosal surfaces of the lungs. Systemic spread of the bacterium is rare. This suggests that the systemic host defense in patients with CF provides adequate protection against bacterial invasion whereas local immunity is overwhelmed. *Staphylococcus aureus* and *Haemophilus influenzae* frequently cause early lung infection in CF. However, death for 90% of CF patients is associated with chronic, persistent colonization and infection with *P. aeruginosa.*

Several studies of the sera of CF patients suffering from chronic *P. aeruginosa* lung infection demonstrated the presence of specific humoral antibodies to the extracellular enzymes alkaline protease (5, 6), elastase (5, 20), and exotoxin A (20) and other antigens, including alginate (28), produced by the pathogen (15). The majority of CF strains produce a slime layer of polyuronic acids (29) in which *P. aeruginosa* persists as microcolonies (22). There is a good correlation between the severity of the lung disease and *P. aeruginosa* antibody titers. This suggests that an exaggerated immune response to *P. aeruginosa* is associated with pulmonary damage in patients with CF (25, 38). In nearly all chronically infected patients, the immune response to *P. aeruginosa* antigens increases for years, suggesting that the production of these antigens is unimpaired during persistent infection. The antibody titers reach a plateau and rarely decline thereafter, suggesting that production of the bacterial antigens and thus stimulation of the humoral immune system are continuous (5, 8).

The high percentage of patients with serum antibodies specific for elastase and/or alkaline protease (83 to 88%) (9) indicates that in vivo production of these enzymes is common in patients with CF. These enzymes may therefore contribute to the pathogenesis of the disease. The roles of exotoxin A and elastase in the virulence of *P. aeruginosa* in chronic lung infections of rats has been demonstrated (39). Amitani et al. (1) suggested that neutrophil elastase and *P. aeruginosa* elastase contribute to the delayed mucociliary clearance and epithelial damage observed in patients with chronic bronchial infection. Thus, *P. aeruginosa* does not merely harmlessly colonize the surface of the lungs of patients with CF. However, the reasons why colonizing *P. aeruginosa* becomes pathogenic in CF patients and the mechanisms behind its pathogenicity are unclear. A detailed understanding of the pathogenesis of exacerbation during chronic *P. aeruginosa* infection may therefore contribute to the development of preventive and curative strategies that prolong and improve the quality of life of CF patients.

The purpose of the present study was to follow the secretion of elastase, exotoxin A, and alkaline protease in sputum during bronchopulmonary exacerbations in patients chronically infected with *P. aeruginosa* and to determine whether or not there is a correlation between the level of these exoproteins and the exacerbation periods in CF.

MATERIALS AND METHODS

Patients. Eighteen CF patients (6 females and 12 males) at ages of from 11 to 28 years old (mean, 19.8 ± 5) who were hospitalized in the Pediatric Department of Hôpital R. Sabran (Hyères, France) and who were chronically infected with *P. aeruginosa* were studied. The diagnosis of CF was based on accepted criteria, including a typical history of CF with significantly high sweat electrolyte levels in repeated tests and altered pulmonary function. The Shwachman scores for the patients were 52 to 78 (mean, 63).

The baseline for chronic *P. aeruginosa* lung infection was defined as the date of collection of the first in an unbroken series of six consecutive monthly samples scoring positive for *P. aeruginosa* (13). Exacerbation was assessed by clinical symptoms (dyspnea, loss of appetite, weight loss, and productive cough) and radiological and bacteriological findings. Patients were studied for one or several exacerbation periods necessitating hospitalizations of 12 to 20 days. The protocol

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was accepted by the institutional human subjects committee, and informed consent was given by all patients or their parents.

Treatment included physiotherapy and antibiotherapy. All patients received antibiotherapy during periods of hospitalization as indicated by the sensitivity of the cultured organism. In most cases, the antibiotherapy was a combination of intravenous tobramycin and cephalosporin. A total of 32 courses of intravenous therapy were given. Ten patients were studied during one exacerbation; eight patients were studied during two or more exacerbations, and three of these were also studied during the interexacerbation bronchial phase when they were hospitalized for digestive disease. Two patients were seen as outpatients. None of the patients received any antiinflammatory drugs or inhaled DNase. All patients inhaled mucolytic agents and antibiotics (colimycin or tobramycin).

Four non-CF patients (two females and two males) aged 20 to 50 years old and one CF patient aged 7 years old infected with *S. aureus* were studied as controls; none of them had *P. aeruginosa* in their sputa.

Collection of bronchial secretions. Sputum collections were made in the morning during chest physiotherapy every 6 days from the onset of acute exacerbation (day 0 of hospitalization) through to posttreatment recovery (day 12 or more). Aerosolized antibiotics were given each afternoon. Sputa were stored at −80°C for 1 week to 3 months before exoproteins were measured.

Bacteriology. Freshly collected sputum was diluted 1:1 (wt/wt) in 2,3-dihydroxydithiolbutane (Digest-Eur; Eurobio Laboratories) diluted 1:10 in distilled water and agitated for 30 min at room temperature.

P. aeruginosa and *S. aureus* bacterial counts were determined by diluting the sputum dilutions (1/2, 1/200, and 1/2,000) in sterile 0.9% NaCl and plating them onto Drigalski and Chapman agar.

Bacteria were identified by plating them on the following media: Drigalski, Chapman, ANC blood agar, chocolate agar, Sabouraud dextrose agar with and without cycloheximide (Acti-Dione), and *Pseudomonas cepacia* OFPBL agar (9.4 g of oxidation-fermentation basal medium [Difco Laboratories], 15.0 g of Bacto agar [Difco], 10.0 g of lactulose [Difco], 300,000 U of polymyxin B sulfate [Sigma], and 200 U of bacitracin [Sigma]).

Immunoenzymatic assays (enzyme-linked immunosorbent assays [ELISA]) in expectorates. After they were thawed, the sputa were homogenized by ultrasonic treatment. Small volumes (2 or 3 ml) of sputum were sonicated in conical tubes for 3 min on crushed ice in a slight modification of the method described by Girard et al. (10). The sputa were then diluted 1:2 (wt/wt) in 0.07 M phosphate-buffered saline (pH 6.0) containing 0.5 M NaCl and 1 U of DNase (Boehringer) per ml. The mixture was stirred for 4 h at 4°C.

The amounts of elastase, exotoxin A, and alkaline protease present were determined by direct double antibody sandwich assays as described elsewhere (17, 18). Monospecific polyclonal antibodies against *P. aeruginosa* elastase, alkaline protease, and exotoxin A were prepared by the immunization of rabbits. Purified *P. aeruginosa* elastase and alkaline protease were obtained from Nagase, Osaka, Japan, and purified exotoxin A was obtained from the Swiss Serum and Vaccine Institute, Bern, Switzerland. ELISA tests were performed in duplicate in coated wells on both homogenized bronchial expectorates and their supernatants, which were obtained by centrifugation.

Immunoenzymatic assays (ELISA) in culture supernatants. *P. aeruginosa* strains were isolated from sputum on *Pseudomonas* isolation agar. Several colonies (five to six representative of the bacterial population were chosen and subcultured in 3 ml of Trypticase soy broth enriched with 0.5% yeast extract and 0.5% glucose. The culture was shaken for 6 h at 30°C with maximal aeration. Then, as described elsewhere (18), 1-ml aliquots of this culture adjusted to a final density of 5.10^8 bacteria per ml were each added to 9 ml of (i) Trypticase soy broth medium for elastase and alkaline protease assays and to 9 ml of (ii) Trypticase soy broth-chelex 100 medium for exotoxin A assays. The cultures were shaken again as described above (for a period of 18 h). The cultures were centrifuged (10,000 × *g*) for 10 min at 4°C, and the frozen supernatants were stored at −80°C until they were used for ELISA.

Determination of serum inflammatory and nutritional markers. Inflammatory markers (C-reactive protein, orosomucoid, and haptoglobin) and nutritional markers (prealbumin and retinol-binding protein) were measured in sera by immunoturbidimetry (Cobas-Mira; Roche Diagnostica).

Statistical analysis. All the data are expressed as median values over interquartile ranges.

The nonparametric Wilcoxon *t* test was used to compare the bacteria load (CFU) and the exoprotein levels at admission and after therapy. Samples were handled as paired samples.

The simple regression test was used to analyze the relationships among the different exoproteins and among exoprotein levels in sputa and in culture supernatants. The nonparametric Spearman correlation test was used to relate the bacteria load (CFU) to the exoprotein levels in individual patients.

A *P* value of <0.05 was considered significant.

RESULTS

Bacteriology of the sputa. Bronchial secretions collected from all patients at admission contained *P. aeruginosa*. *P. aeruginosa* was similarly detected in the bronchial secretions of all patients after treatment.

TABLE 1. Microorganisms associated with *P. aeruginosa* during 29 episodes of exacerbation

Microorganism	No. of isolations	
	Admission	End of treatment
Staphylococcus aureus[a]	11	1
Alcaligenes xylosoxidans subsp. *xylosoxidans*[a]	4	0
Haemophilus influenzae	1	0
Mycoplasma pneumoniae	1	0
Streptococcus haemolyticus (group C)	1	0
Aspergillus sp.	3	2
Scedosporium sp.	1	1

[a] Cases in which bacteria loads were greater than 10^5 CFU/g of sputum.

Twenty-nine episodes of bronchopulmonary exacerbation were studied. One or two other microorganisms which were associated with *P. aeruginosa* were isolated at admission for 20 of these episodes. As can be seen from Table 1, the associated organisms (except for fungi) were not found or were found at concentrations lower than 10^5 CFU/g at the end of the treatment. The most frequently associated pathogen was *S. aureus*, which was present during 11 episodes (38% of the exacerbations). The other pathogens were *Alcaligenes xylosoxidans* subsp. *xylosoxidans* (four times), *H. influenzae* (once), *Mycoplasma pneumoniae* (once), *Streptococcus haemolyticus* (group C) (once), and the filamentous fungi *Aspergillus* sp. and *Scedosporium* sp. (four times).

The density of *P. aeruginosa* at admission was always more than 10^6 CFU/g, with a mean of 3.18×10^7 CFU/g (Fig. 1). After treatment, except in six cases of exacerbation, the count remained high (above 10^6 CFU/g). During three episodes of nonpulmonary infection, the CFU densities at admission were also above 10^6 CFU/g.

Detection of exoproteins in homogenized sputa. To ensure accurate and representative measurements of the exoproteins in the sputa, the samples were liquified and homogenized. We tested different methods: a chemical method using a mucolytic agent (dithiothreitol) and a mechanical method using ultrasound in the presence of DNase. The DNase-sonication treat-

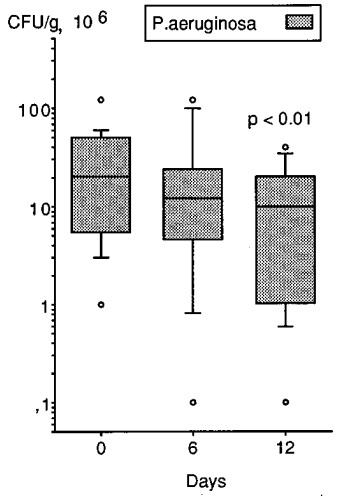


FIG. 1. Box plots of bacterial counts of *P. aeruginosa* (in CFU per gram) in sputa of 18 CF patients during 29 bronchopulmonary exacerbations before (day 0), during (day 6), and after (day 12) treatment. The statistical significance of the day 0 and day 12 values by the Wilcoxon paired *t* test was $P < 0.010$.

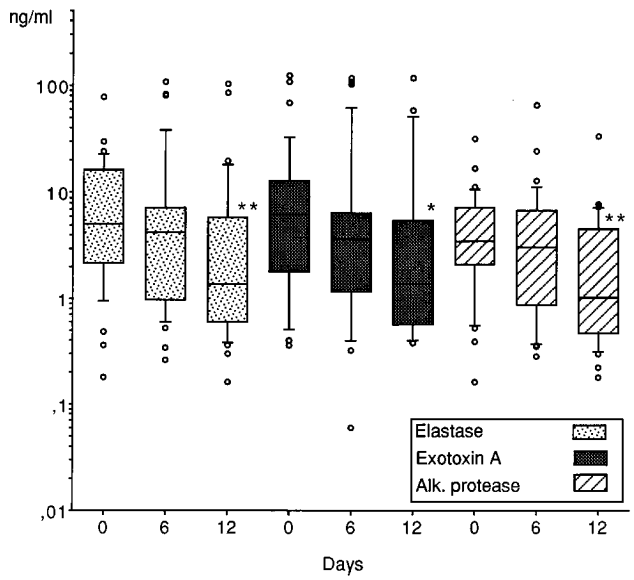


FIG. 2. Box plots of levels of *P. aeruginosa* exoproteins elastase, exotoxin A, and alkaline protease in total homogenized sputa during 29 bronchopulmonary exacerbations before (day 0), during (day 6), and after (day 12) treatment. The statistical significances of the day 0 and day 12 values by the Wilcoxon paired *t* test were $P < 0.01$ for elastase,** $P < 0.05$ for exotoxin A,* and $P < 0.01$ for alkaline protease.**

ment appeared to be more reproducible and more practicable and was therefore used as detailed in the Materials and Methods section.

The next step was to determine which fraction to use for the ELISA: total homogenized sputum or supernatant of homogenized sputum after it was centrifuged at low or high speed (8,000 or 18,000 × *g*). Two different sputum samples were assayed for this purpose. The amounts of elastase detected in the supernatants (1.32 and 1.32 ng/ml) were lower than in those in the total homogenized sputa (2.52 and 2.45 ng/ml). Presumably, exoproteins are trapped in the mucus and were therefore not detected by immunoassays of the supernatants. Pellets proved unsatisfactory because of high irreproducibility. Thus, total homogenized sputum samples were used for all ELISA.

Exoproteins were detected in all sputum specimens taken at admission, albeit at low levels. The amounts detected varied significantly among patients: from 0.3 ng/ml to 79 ng/ml for elastase, from 0.3 ng/ml to 126 ng/ml for exotoxin A, and from 0.3 ng/ml to 32 ng/ml for alkaline protease (Fig. 2). However, in a given patient, the amounts of the three proteins were correlated (data not shown) (elastase-exotoxin A, $r = 0.87$; elastase-alkaline protease, $r = 0.67$; exotoxin A-alkaline protease, $r = 0.68$; $P < 0.001$ in all cases).

Several controls were performed. The levels of exoproteins in five samples collected from patients during intercrisis phases were extremely low, less than 1 ng/ml. Three of these patients were studied during hospitalizations justified by digestive reasons (abdominal pain and occlusive abdominal syndrome), and two of them were controlled on the occasion of a consultation in the outpatient department. Sputum from a CF patient not infected with *P. aeruginosa* but infected with *S. aureus* was also analyzed; it was negative for the three exoproteins. Sputa from four non-CF patients not infected with *P. aeruginosa* were also negative for the three exoproteins. The absence of interference with neutrophil elastase was verified with a homogenate of granulocytes originating from a sample of septic urine.

Time course of production of exoproteins during exacerbations. We followed the levels of elastase, exotoxin A, and alkaline protease in sputum during the treatment of 29 episodes. The mean levels of production of the three exoproteins were significantly lower after 12 days of antibiotherapy than before treatment (Fig. 2). A clinical improvement was observed in all cases after treatment, correlating with the evolution of inflammatory and nutritional markers which returned to a normal range (data not shown) after the course of antibiotics.

There were large differences among the time courses of production in different patients and among exacerbation episodes in the same patient. In the majority of the 29 episodes of exacerbation, the production of exoproteins followed a classical pattern, with high levels at admission and a decrease after 12 days of antibiotherapy. However, four cases did not conform to this pattern and showed variable increasing levels which could not be correlated to the clinical parameters. During eight exacerbation episodes, patients needed a modification of the antibiotherapy, generally implemented at day 6. The therapeutic adaptation was followed in the majority of these cases by a decrease in exoprotein levels.

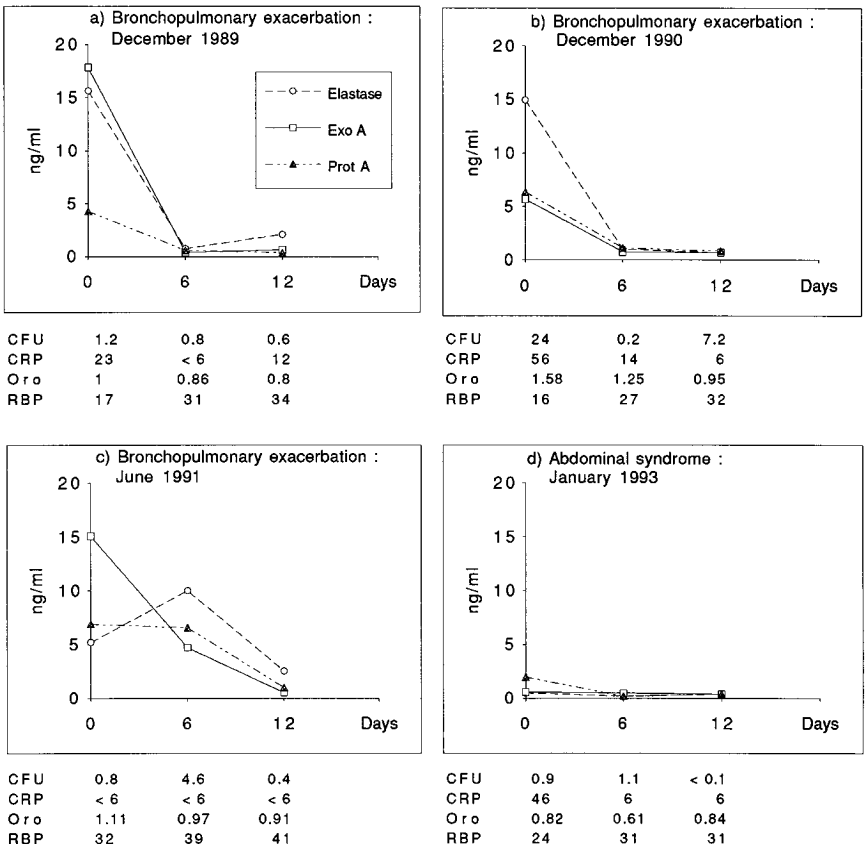
Exoprotein levels in the sputum of a patient hospitalized four times are represented in Fig. 3. During the first three hospitalizations, which were due to bronchopulmonary exacerbation, the kinetics were similar, with the secretion levels of the exoproteins before treatment running at about 10 ng/ml and decreasing to 2 ng/ml after treatment. During the fourth hospitalization, which was due to an abdominal syndrome, the levels of exoproteins were extremely low, less than 1 ng/ml.

The results obtained in two patients hospitalized for a bronchopulmonary exacerbation are illustrated in Fig. 4. In these two cases, *P. aeruginosa* was associated in sputum with *A. xylosoxidans* subsp. *xylosoxidans*. At day 6 for the first patient and at day 12 for the second patient, *A. xylosoxidans* subsp. *xylosoxidans* alone was detected ($>10^6$ CFU/g) on agar plates, and this coincided with low levels of exoproteins. At day 12 and day 18, *P. aeruginosa* and an associated increase in the exoprotein levels were detected.

The results from three patients hospitalized for a bronchopulmonary infection for which the first antibiotherapy was not effective are represented in Fig. 5. The antibiotherapy was then modified on day 6 on the basis of the sensitivity of the bacteria in vitro. Exoprotein production was lower after the modification of the treatment.

Exoproteins in culture supernatants of strains isolated from sputa. In an attempt to characterize the secretory phenotypes of the bacteria present in the various expectorates, we isolated several colonies from several samples and determined the amounts of exoproteins secreted after one subculture in liquid medium. Results from one such expectorate are shown in Table 2. Five isolated colonies, two mucoid and three nonmucoid, were subcultured separately. They produced very different amounts of exoproteins in the same standard conditions of culture. Thus, the bacterial population in the sputum of cystic fibrosis patients is heterogeneous.

In another set of experiments, we pooled for every sputum five to six colonies which appeared morphologically representative of the bacterial population. After one subculture in liquid medium, the exoproteins were assayed. The amounts were very variable, ranging from 0.3 ng/ml to 3 μg/ml for elastase, from 0.3 ng/ml to 120 ng/ml for exotoxin A, and from 0.3 ng/ml to 5 μg/ml for alkaline protease. There was a close correlation in the production of the three exoproteins in these subcultures: elastase-exotoxin A, $r = 0.59$; elastase-alkaline protease, $r = 0.66$; exotoxin A-alkaline protease, $r = 0.71$; $P < 0.001$ in all



CFU	1.2	0.8	0.6
CRP	23	< 6	12
Oro	1	0.86	0.8
RBP	17	31	34

CFU	24	0.2	7.2
CRP	56	14	6
Oro	1.58	1.25	0.95
RBP	16	27	32

CFU	0.8	4.6	0.4
CRP	< 6	< 6	< 6
Oro	1.11	0.97	0.91
RBP	32	39	41

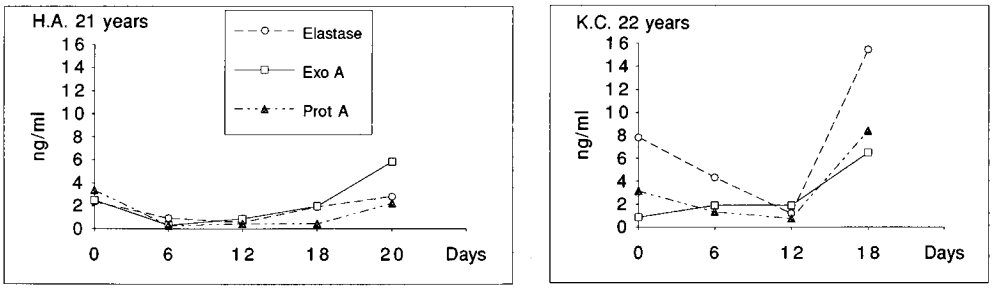
CFU	0.9	1.1	< 0.1
CRP	46	6	6
Oro	0.82	0.61	0.84
RBP	24	31	31

FIG. 3. Time courses of elastase, exotoxin A, and alkaline protease levels in sputum of a patient during successive hospitalizations: three hospitalizations for bronchopulmonary exacerbation (a, b, and c) and one hospitalization for an abdominal syndrome (d). CFU, *P. aeruginosa* bacteria count (10^6 CFU/g); CRP, C-reactive protein serum level (milligrams per liter); Oro, orosomucoid serum level (grams per liter); RBP, retinol-binding protein serum level (milligrams per liter).

cases. However, there was no correlation between the levels of exoproteins in sputa containing the strains and the levels of secretion when subcultured.

DISCUSSION

The condition of CF patients hospitalized for treatment of an exacerbation of their pulmonary disease improves after 10 to 14 days of intravenous antibiotic therapy associated with vigorous chest physiotherapy. However, the contribution of the antibiotics themselves to the clinical response is controversial (2, 16). Some authors (11, 12) did not find significant differences in *P. aeruginosa* density in sputum after antibiotherapy. This was not confirmed by other groups, which reported significant decreases in sputum *P. aeruginosa* density after therapy



	P.aer	Alc	P. aer & Alc	P. aer & Alc	P. aer
CFU	18	12	6	5	-
CRP	19	88	61	16	15
Oro	1.52	1.75	1.7	1.35	1.61
RBP	15	15	17	33	42

	P. aer & Alc	P.aer & Alc	Alc	P.aer & Alc
CFU	40	19	16	40
CRP	132	56	17	63
Oro	2.51	2.58	1.98	1.71
RBP	15	29	33	29

FIG. 4. Time courses of exoprotein production in sputa of two patients colonized with *A. xylosoxidans* subsp. *xylosoxidans* (Alc) and *P. aeruginosa* (P. aer). CFU, bacteria count (10^6 CFU/g); CRP, C-reactive protein serum level (milligrams per liter); Oro, orosomucoid serum level (grams per liter); RBP, retinol-binding protein serum level (milligrams per liter).

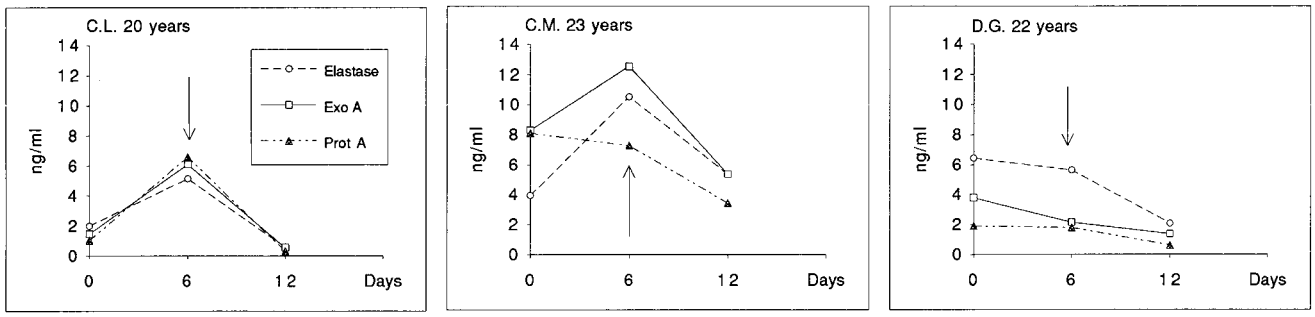


FIG. 5. Effects of modification of the antibiotherapy on the time courses of the production of exoproteins in sputa during exacerbations for three different patients. The arrows indicate the modification of the antibiotic treatment. CFU, bacteria count (10^6 CFU/g); CRP, C-reactive protein serum level (milligrams per liter); Oro, orosomucoid serum level (grams per liter); RBP, retinol-binding protein serum level (milligrams per liter).

(33, 34). The correlation, if any, of clinical status, antibiotic susceptibility, and organism densities remains unclear (23, 27). *P. aeruginosa* colony counts do not appear to be a reliable marker of bronchopulmonary exacerbation: the density of bacteria after antibiotherapy remains in the range of that corresponding to infection, i.e., more than 10^6 CFU/g of sputum (23), and thus cannot be used to differentiate acute exacerbation from colonization. The results obtained in our study confirmed these data: in 29 episodes of exacerbation, the bacterial density decreased significantly after therapy (Fig. 1), although it remained higher than 10^6 CFU/g of sputum in most cases.

P. aeruginosa elastase, alkaline protease, and exotoxin A were found in small amounts in all sputa. These small amounts of exoproteins are evidence for secretion in vivo. Their variations suggest an increased virulence of *P. aeruginosa* during exacerbations. Exoprotein levels were high at admission, significantly decreased after antibiotherapy, and absent during intercrisis phases. Thus, exoprotein levels correlated inversely with clinical improvement in chronically infected patients. This also argues for the contribution of antibiotics to the improvement of CF pulmonary exacerbations. In spite of the fact that the secretion of the three exoproteins was well correlated, there were few cases in which one of the three exoproteins was not detected. However, the virulence of *P. aeruginosa* is multifactorial, and the absence of some of the virulence factors may not necessarily affect the ability of the bacteria to colonize and persist in the lung (26).

Our results are in perfect agreement with the results obtained by Rabin et al. (32), Storey et al. (35), and Grimwood et al. (11) by completely different experimental approaches. Measuring the mRNA accumulation patterns in sputum, Storey et al. (35) suggested that *algD*, *lasB*, and *toxA* transcript accumulation correlated with disease severity and that antibiotherapy (when resulting in clinical improvement) suppressed this accumulation. On the other hand, comparisons of in vitro exoenzyme production by *P. aeruginosa* sputum isolates showed that antibiotics improved pulmonary function by decreasing *P. aeruginosa* exoprotein production rather than by reducing bacterial numbers (11).

TABLE 2. Culture supernatant levels of exoproteins produced by different colonies isolated from the same patient

Colony (type)	Amount (μg/ml)		
	Elastase	Exotoxin A	Alkaline protease
Colony 1 (nonmucoid)	65	29.3	26.5
Colony 2 (nonmucoid)	80	19.6	3.09
Colony 3 (mucoid)	116	20	15.3
Colony 4 (mucoid)	14.1	11.2	0.067
Colony 5 (mucoid)	8.86	9.5	0.008

The heterogeneity and variability of *P. aeruginosa* isolates in the pulmonary flora of chronically infected CF patients have been recently demonstrated (4). The coexistence of colonies with different serotypes and other biological characteristics has also been reported in clinical isolates of *P. aeruginosa* (21). The levels of the exoproteins are thus the result of the contributions of different subpopulations. This heterogeneity of the bacterial population may indicate that the important factor is not bacterial proliferation per se but rather the virulence of a part of the population during the exacerbation. Under particular conditions, some more-virulent bacterial populations could prevail and the amounts of the exoproteins in the sputum could therefore change. Thus, a renewal of virulence by a part of the population responsible for the secretion of exoproteins, rather than an increase in bacterial density, could explain the modifications in exoprotein levels detected during exacerbations.

The production of exoproteins by subcultures of freshly isolated *P. aeruginosa* strains does not seem to correlate with the in vivo production of exoproteins. Indeed, the secretions from mucoid and nonmucoid strains from a given patient were quite different (Table 2). Moreover, the preparation of the inoculum for subcultures is very subjective, and it is difficult to collect colonies truly representative of the overall bacterial population. This may explain the absence of correlation. Another explanation for the lack of correlation between the expression of exoproteins under laboratory conditions and that observed in vivo is that this expression is probably influenced by the environmental and nutritional conditions that the bacteria find. These conditions may be sufficiently different in bronchial secretions and in vitro to account for alterations in exoprotein production.

Some authors report that *P. aeruginosa* exoproteins do not have a direct deleterious effect in chronic lung injury in CF. They suggest that the inflammatory process induced by the presence of immune complexes was mainly responsible for the pathogenesis (3, 7, 14). Nonetheless, this still implicates *P. aeruginosa* exoproteins, as they could maintain the production of immune complexes. Moreover, it has been shown that *P.*

aeruginosa elastase inactivates two protease inhibitors, the bronchial mucosal proteinase inhibitor (19) and α1-proteinase inhibitor (24, 36), which are major inhibitors of polymorphonuclear elastase. This may contribute to the imbalance of proteases and protease inhibitors which characterizes bronchial inflammation in CF (37).

We found other infectious agents in 69% of the exacerbations. A synergism between other microorganisms (*Mycoplasma* and *Chlamydia* spp.) and *P. aeruginosa* has been suggested (30, 31). The evaluation of the role played by *P. aeruginosa* in the onset of acute bronchopulmonary exacerbation is complicated by the concomitant presence of other infectious agents. Our study underlines the complexity of both infectious bronchopulmonary disease during CF and its management.

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